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A topological function based on spectral moments for predicting affinity toward A_3 adenosine receptors

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Abstract—The spectral moment descriptors have been applied to the study of affinity for A_3 adenosine receptors of 32 adenosine analogues. A model, able to describe more than 95% of the variance in the experimental activity, was developed with the use of the above-mentioned approach. The fragment contributions to the activity carried out show that the sulfonamido moiety at the N^6 position and hydrogen bonding play an important role in the interaction with the receptor.

The adenosine receptors (ARs) consist of four subtypes (A_1, A_{2A}, A_{2B}, and A_3) and represent a physiologically important family of G protein-coupled receptors.[1] The A_3AR is the youngest member of the AR family and the last to be cloned.[2] This receptor subtype shows more species differences than other AR subtypes between rodents and humans in amino acid sequence, ligand binding affinity (particularly antagonist), and tissue distribution. In humans, the highest A_3 receptor densities are found in lung, liver, and cells of the immune system.[3] For that reason this receptor has been closely related to several diseases such as cancer, inflammation, cardiac ischemia, and cerebral ischemia, and it has been a promising target for the development of new therapeutic agents.[4]

Although a large number of adenosine derivatives have been tested at the adenosine A_3 receptor, only a few of them are known as potent and selective agonists.[3] These selective agonists at A_3AR have been obtained by optimization of substituents at N^6, C2, and C5′ of adenosine.[4] In this sense, useful tools are necessary to find a new selective agonist.

Quantitative structure–activity relationship (QSAR) studies are a powerful method for the design of bioactive compounds and prediction of activity according to physical and chemical properties.[5–11] Nevertheless, this technique, on especially the spectral moments, has not been applied to the A_3AR. Only one report in this connection appears in the literature[12] and few researches using mutagenesis and molecular modeling studies have been carried out in this way.[3]

For that reason, in this research we have tried to describe the QSAR between the A_3AR and a set of 5′-*N*-ethylcarboxamido analogues of adenosine (adenosine-5′-ethyluronamide derivatives) structurally related to Cl-IB-MECA, the current standard A_3 agonist. For this propose we used the spectral moments implemented in the TOPS MODE software as molecular descriptors because of the successful application of this theoretical approach in the modeling of several biological activities and their possibilities of discovery alert structures that should be used in the synthesis of new adenosine analogues.[5,13–15] In addition, the best model according to this approach was compared with several models obtained using different methodologies reported by us recently.[16]

The spectral moments are based on the calculation of bond matrix, whose theoretical basis has been widely described in previous reports.[17–19] Essentially, the bond matrix is the square and symmetric matrix whose entries are ones or zeros according to whether the corresponding bonds are adjacent or not. The order of this matrix (m) is the number of bonds in the molecular graph, being two bonds adjacent if they are incident to a com-

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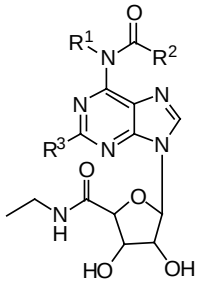
mon atom. The spectral moments of the edge adjacency matrix are defined as the traces, i.e., the sum of the main diagonal of the different powers of such a matrix.

In order to apply the above approach to the development of a model for predicting affinity at A_3ARs, the following steps were followed: (a) an adequate training set of chemicals was selected; (b) the molecular graphs for each molecule of the training set were drawn; (c) the molecular bonds with appropriate weights were differentiated (standard distance, hydrophobicity, and van der Waals atomic radii), the spectral moments calculated, and the QSAR model found using multiple regression analysis; (d) the predictive performance of the model was assessed by the leave-one-out (LOO) and leave-group-out (LGO) cross-validation methods; and (e) the contribution of the different fragments was determined in order to know of their quantitative contribution to the biological activity of the molecules studied.

Here, we have selected a data set of 32 adenosine analogues, whose affinity at A_3 receptors was reported.[20] The affinity of these compounds was measured by displacement of specified [^{125}I]AB-MECA binding at rat A_3 receptors expressed in Chinese hamster ovary (CHO) cells, given as K_i in nM. The experimental values of this property are given in Table 1.

Note that the compounds **9** and **10** in this training set present a chiral center and are stereoisomers. The TOPS-MODE approach does not differentiate between these compounds because it takes into account only

Table 1. Structures and affinities of compounds in radioligand binding assays at rat A_3 adenosine receptors expressed in CHO cells, used in the current work



Compound	R^1	R^2	R^3	$K_i(A_3)$[a] nM
1	H	4-Biphenyl	H	979
2	H	2,4-Cl-Ph-CH_2	H	167
3	H	4-CH_3O-Ph	H	837
4	H	2-Cl-Ph	H	754
5	H	Ph	H	824
6	H	$PhCH_2NH$	H	38.3
7	H	4-NH_2SO_2PhNH	H	9.73
8	H	4-CH_3CO-PhNH	H	20.9
9	H	(*R*)-α-Phenylethyl-NH	H	16.3
10	H	(*S*)-α-Phenylethyl-NH	H	319
11	H	5-Me-Isoxazol-3-yl-NH	H	532
12	H	1,3,4-Thiadiazol-2-yl-NH	H	5550
13	H	4-*n*-C_3H_7O-PhNH	H	107
14	H	Ph-CH_2CH_2NH	H	149
15	H	3,4-MeO-Ph-CH_2CH_2NH	H	411
16	H	Fur-2-yl-CH_2NH	H	713
17	H	4-(Pyridin-2-yl-$NHSO_2$)PhNH	H	54.1
18	H	4-(5-Me-Isoxazol-3-yl-$NHSO_2$)PhNH	H	155
19	H	4-(Pyrimidin-2-yl-$NHSO_2$)PhNH	H	405
20	4-NO_2-Ph-NH-CO	4-NO_2-Ph-NH	H	168
21	5-Cl-Pyridin-2-yl-NH-CO	5-Cl-Pyridin-2-yl-NH	H	5700
22	H	3-Cl-Ph-NH	Cl	77.6
23	H	4-MeO-Ph-NH	Cl	17.1
24	H	3-Cl-Ph-NH	I	315
25	H	4-MeO-Ph-NH	I	251
26	H	3-Cl-Ph-NH	*n*-C_4H_9-C≡C	581
27	H	3-Cl-Ph-NH	Ph-C≡C	611
28	H	3-Cl-Ph-NH	PhCH(OH)-C≡C	696
29	H	4-MeO-Ph-NH	*n*-C_4H_9-C≡C	211
30	H	4-MeO-Ph-NH	Ph-C≡C	154
31	H	4-MeO-Ph-NH	PhCH(OH)-C≡C	324
32	H	4-MeO-Ph-NH	$Ph(CH_2)_3$-C≡C	89.5

[a] Displacement of specified [^{125}I]AB-MECA binding at rat A_3 receptors expressed in CHO cells, expressed as K_i in nM (n = 3–6).

the 2-D information of these. However, we decided not to eliminate any of these due to the small number of adenosine analogues in the training set.

TOPS MODE[21] computer software was employed to calculate the molecular descriptors. The standard distance, hydrophobicity, and atomic van der Waals radii were used as bond weightings for making differentiation of heteroatoms. So, three sets of spectral moments were obtained.

In general, 15 spectral moments were calculated for each of the studied schemes. We also used multiplication of spectral moments as independent variables for describing the affinity for A_3 receptors on these kinds of compounds. In this case, we multiplied μ_0 and μ_1 for the first 10 spectral moments obtaining 20 new variables, because of useful information included in these new variables, which make a total number of 75 descriptors.

The most significant parameters were identified from the data set using genetic algorithm (GA) analysis of the descriptors obtained by TOPS MODE computer software. The GA is a class of methods based on biological evolution rules. The first step is to create a population of linear regression models. These regression models mate with each other, mutate, cross-over, reproduce, and then evolve through successive generations toward an optimum solution. The GA simulation conditions were 10000 generations and 300 populations. The models were linear combinations of six descriptors. The GA procedure was repeated n times to confirm that the selected descriptors are the optimal descriptor set for describing the modeled property. Examining the regression coefficients, the standard deviations, the significances, and the number of variables in the equation, we determined the quality of the models. All statistical analysis and data exploration were carried out using the Statistica 6.0.[22]

The models obtained were validated by calculating q^2 values. The q^2 values are calculated from LOO and LGO tests known as cross-validation; in this last case, 25% of the data was selected for the analysis. The q^2 values can be considered a measure of the predictive power of a regression equation: whereas R^2 can always be increased artificially by adding more parameters (descriptors), q^2 decreases if a model is overparameterized and is therefore a more meaningful summary statistic for QSAR models.

In this sense, the best QSAR model obtained with the spectral moment descriptors is given below together with the statistical parameters of regression:

$$-\log K_i = -10.149 + 0.002 \cdot \mu_0\mu_4^{dist} - 6.164 \cdot 10^{-8} \cdot \mu_1\mu_{10}^{dist} - 0.005 \cdot \mu_0\mu_4^{Hyd} + 6.771 \cdot 10^{-6} \cdot \mu_0\mu_9^{Hyd} + 0.029 \cdot \mu_3^{VDW} - 0.002 \cdot \mu_1\mu_2^{VDW}, \quad (1)$$

$$N = 32; \quad R = 0.912; \quad S = 0.301; \quad F_{exp} = 20.645;$$
$$p < 10^{-5}; \quad q^2_{LOO} = 0.768; \quad S_{LOO} = 0.354;$$
$$q^2_{LGO} = 0.731; \quad S_{LGO} = 0.367.$$

where N is the number of compounds used, R is the correlation coefficient, S is the standard deviation of the regression, F_{exp} is the Fisher ratio at the 95% confidence level, p is the significance of the variables in the model, and q^2_{LOO}, S_{LOO} and q^2_{LGO}, S_{LGO} are the square of the correlation coefficient and the standard deviation of the leave-one-out and leave-group-out cross-validation, respectively.

Although this theoretical model has six variables and acceptable statistical parameters, a step-by-step outlier extraction procedure led to different models with a better statistical profile. In this study, two outliers extracted, considering that a number of outliers lower than 10% of the general data are classically accepted in the literature as threshold limit value for outliers' extraction. In our case, the higher extracted outlier number represented a 6.25% of the whole data. The compounds **6** and **9** present large residuals and should be considered as outliers. On removal of these compounds from the training set, the next equation is obtained:

$$-\log K_i = -11.002 + 0.001 \cdot \mu_0\mu_4^{dist} - 6.019 \cdot 10^{-8} \cdot \mu_1\mu_{10}^{dist} - 0.005 \cdot \mu_0\mu_4^{Hyd} + 6.447 \cdot 10^{-6} \cdot \mu_0\mu_9^{Hyd} + 0.031 \cdot \mu_3^{VDW} - 0.002 \cdot \mu_1\mu_2^{VDW}, \quad (2)$$

$$N = 30; \quad R = 0.975; \quad S = 0.157; \quad F_{exp} = 73.042;$$
$$p < 10^{-5}; \quad q^2_{LOO} = 0.913; \quad S_{LOO} = 0.208;$$
$$q^2_{LGO} = 0.882; \quad S_{LGO} = 0.239.$$

As can be seen the statistical parameters of the equation improved significantly from a statistical point of view, therefore this is the final evidence and compounds **6** and **9** should be considered as potential outliers. In addition, a result of LGO demonstrated that the q^2 of this test ($q^2 = 0.882$) improved the below-mentioned results.

A possible explanation for this behavior may be due to the fact that this variant of the spectral moment does not recognize the enantiomers and for that reason the compound **9** is consider as outlier.

On the other hand, before making the interpretation of the model we need to orthogonalize the molecular descriptors included in such models due to the high intercorrelation existing between some of them.

For that reason, an orthogonalization process is necessary to eliminate this undesirable effect. Here, we used the Randić orthogonalization method to correct this problem.[23–25] The main philosophy of this approach is to avoid the exclusion of descriptors on the basis of its

collinearity with other variables previously included in the model. In view of the authors, the collinearity of the variables should be as low as possible because the interrelatedness among the different descriptors can result in a highly unstable regression coefficient, which makes it impossible to know the relative importance of an index and underestimates the utility of the regression coefficient model.

The QSAR model obtained with the spectral moment after the orthogonalization and standardization process is given below together with the statistical parameters.

$$-\log K_i = -2.426 - 0.319 \cdot \mu_0\mu_4^{dist} - 0.460 \cdot \mu_1\mu_{10}^{dist} - 0.098 \cdot \mu_0\mu_4^{Hyd} + 0.101 \cdot \mu_0\mu_9^{Hyd} + 0.087 \cdot \mu_3^{VDW} - 0.182 \cdot \mu_1\mu_2^{VDW}, \quad (3)$$

$$N = 30; \quad R = 0.975; \quad S = 0.157; \quad F_{exp} = 73.042;$$
$$p < 10^{-5}; \quad q^2_{LOO} = 0.913; \quad S_{LOO} = 0.208;$$
$$q^2_{LGO} = 0.882; \quad S_{LGO} = 0.239.$$

Once the variables of the model have been orthogonalized we can carry out an interpretation of this. As we previously explain, the TOPS-MODE approach is able to computed the contribution of any structural fragment to the biological activity studied. In the present case, we can find the positive and negative contributions of such fragments to the affinity for A_3AR. In Figure 1, we show the structures of a set of selected fragments. The contributions to the activity of these fragments were computed using the Eq. 3. These quantitative contributions are given in Table 2.

Although many interpretations according to these results could be established, we only highlight the more

Table 2. Contribution of selected fragments to the affinity at A_3ARs

Fragment	Contribution
F_1	−0.192
F_2	0.221
F_3	0.108
F_4	−0.029
F_5	−0.557
F_6	−0.069
F_7	−0.704
F_8	−0.351
F_9	−0.320
F_{10}	−0.116
F_{11}	0.081
F_{12}	0.259
F_{13}	0.287
F_{14}	0.424
F_{15}	1.161
F_{16}	0.099
F_{17}	0.533
F_{18}	0.248
F_{19}	−0.518
F_{20}	−0.381
F_{21}	−1.027
F_{22}	−0.580
F_{23}	0.073
F_{24}	−0.004
F_{25}	−0.191
F_{26}	−0.593
F_{27}	−0.659
F_{28}	0.251
F_{29}	0.109
F_{30}	0.397
F_{31}	−0.168
F_{32}	−0.344
F_{33}	−0.145
F_{34}	−0.157
F_{35}	−0.447
F_{36}	−0.077
F_{37}	−0.253
F_{38}	−0.033
F_{39}	0.262

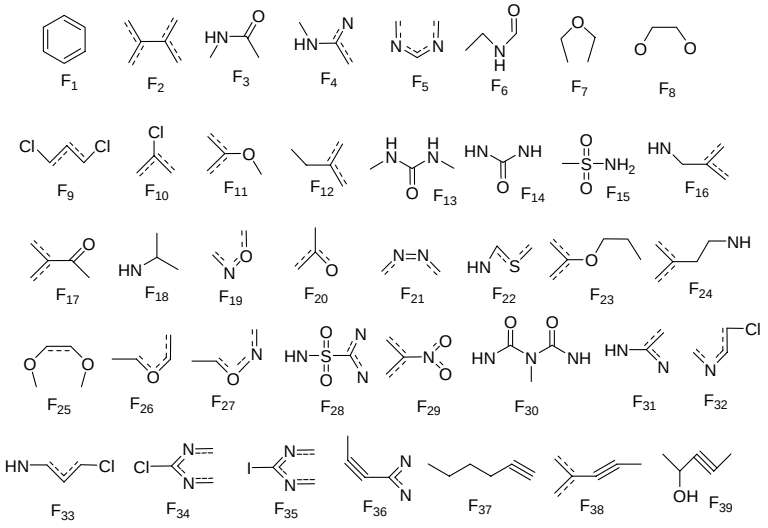


Figure 1. Structures of selected fragments for which their contributions to the affinity at A_3ARs were evaluated.

significant behavior. As can be seen the fragment F_7 possesses a high negative contribution to the affinity for A_3AR in this set of fragments. This implies that the oxygen of the ribose moiety does not improve the affinity of the ligands in this set of compounds and can be substituted. This theoretical conclusion is supported by experimental evidences as reported by Jacobson and co-workers.[26] These authors designed new analogues with high A_3AR affinity that show a modified ribose moiety. These analogues contain the (*N*)-methanocarba(bicyclo[3.1.0]hexane) ring system, which is a rigid ribose substitute lacking the ether oxygen. This ring system maintains the 2′-*exo*-(*N*) ring-twist conformation of the ribose-like ring (pseudosugar moiety), which has been demonstrated to be favored in A_3AR binding (more so than at other AR subtypes). In this connection, these authors found recently that the substitution of the ether oxygen in the ribose moiety by a sulfur atom gives high affinity and selectivity for A_3AR. The finding that the 4′-thio modification is associated with high potency and selectivity significantly expands the possibilities to design anew the A_3AR agonist.[27]

Other negative contributions are shown by the fragments F_{19}, F_{21}, and F_{27}. These fragments only appear in the heterocyclic moiety at the N^6 position in the data set under study. Baraldi et al. reported that the addition of heterocycle moiety in this position leads to derivates with modest affinity at A_3AR.[20] Apparently, the electronic interactions with this type of substitution do not improve the binding of the analogues at the receptor in this position.

On the other hand, the fragment F_3 presents a positive contribution to the affinity for this receptor. This evidence supports the well-established idea that the methyl or ethyl uronamide group in the N^6 position or in the ribose moiety improved the affinity of the analogues in the A_3AR.[3,4,20,26,27]

The fragment F_{14} shows a singular increase in affinity. These findings suggest that the amino group at the N^6 position may be acylated to form a urea with good affinity for the receptor.[28] We think that the additional hydrogen bond donor in the urea moiety might increase the binding in the protein pocket that is occupied for these substituents at the N^6 position in these analogues.

In this connection, many researches claim the idea that the amino group in the N^6 position should not be disubstituted because it would lose the possibility of hydrogen bond interactions and the analogues decrease their affinity for the A_3AR. However, this theory is not very clear because their bases support the idea that at least a hydrogen in the amino group is essential for the affinity. The fragment F_{30} shows that the nitrogen of the original amino group has been functionalized twice, but presents a positive contribution to biological property. The explanation for this interesting behavior could be that the urea hydrogens assume this role in these analogues.

Finally, the fragments F_{15} and F_{28} contain a sulfonamide substructure. These fragments have a clear positive effect on the affinity of analogues under study. Ijzerman group found that the alkylthio substituents increased A_3 affinity and selectivity in all cases in a family of N^6,5′-disubstituted adenosine derivates.[29] In this sense, Baraldi et al.[30] proved that a set of N^6-[4-(substituted)sulfonamidophenylcarbamoyl]adenosine-5′-uronamides present a high affinity at A_3AR in the nanomolar range. All of these molecules displayed high selectivity at A_3AR versus A_1AR. For that reason, we suppose that the electronic properties of the sulfonamido moiety play an important role in the interaction with the receptor at the N^6 position.

These examples show that the spectral moments can be a potential tool for the design of new adenosine analogues with desirable properties based on the fragment contribution.

Finally, for demonstrating the superiority of this methodology we compared this with those of others previously reported by us.[16] For this aim, the spectral moments were compared with those of BCUT descriptors that present the best characteristic from a statistical point of view of the models reported recently.[16]

The statistical information for the best regression of affinity for A_3 adenosine receptors with these molecular descriptors show that the spectral moments explain the experimental variance of the data better than the BCUT approximation using the same number of descriptors in the equation. Also it presents the best validation parameters, regarding the better predictive power as the reason why they demonstrate their superiority.

In this sense, additional criterions exist to compare the quality of different models. One of these criterions was formulated by Akaike some time ago.[31,32] Akaike's information criteria (AIC) take into account the statistical goodness of fit and the number of parameters that have to be estimated to achieve that degree of fit. This criterion is calculated using the following equation:

$$\mathrm{AIC} = \mathrm{RSS} \cdot \frac{(n + p')}{(n - p')^2}, \tag{4}$$

where RSS is the sum of squared differences between the observed (y) and estimated response (y'), n is the number of compounds in the training set, and p′ is the number of adjustable parameters in the model. When comparing models, the model that produces the minimum value of these statistics should be considered potentially the most useful. We calculated the Akaike values for the two approaches and included these in the comparison, demonstrating again the superiority of the TOPS-MODE approach (AIC = 0.141) over the BCUT descriptors (AIC = 0.161).

The prediction of affinity for A_3AR of adenosine analogues has been a goal of pharmaceutical companies and different laboratories due to the importance of this biological property for many pharmacological targets. In this connection, the linear model developed in the current work is easily calculated, suitable for the rapid

prediction of A_3AR affinity, and the statistical parameters and the cross-validation of the final model support this claim. In addition, the possibility of development of fragment contribution of the analogues to the property suggests that this method can be regarded as one clear choice for lead optimization programs in the drug discovery process.

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